



DEFENSE-RELATED ENZYMES IN PLANT DISEASE RESISTANCE: MOLECULAR REGULATION AND AGRICULTURAL APPLICATIONS

Fatma Gönül SEZGİN^{1*}

¹Ondokuz Mayıs University, Faculty of Science, Department of Biology, 55139, Samsun, Türkiye

Abstract: Plant diseases caused by fungal, bacterial, and viral pathogens represent a major constraint to global agricultural productivity. Plants rely on a complex and highly regulated defense system in which defense-related enzymes play central roles in restricting pathogen invasion and disease progression. This review provides a comprehensive overview of the enzymatic components of plant disease resistance, with a particular focus on key defense-related enzymes, including phenylalanine ammonia-lyase, peroxidases, chitinases, β -1,3-glucanases, and polyphenol oxidase. The biochemical functions of these enzymes in reinforcing cell walls, generating antimicrobial compounds, and regulating reactive oxygen species are discussed in relation to their contribution to plant immunity. Furthermore, the molecular regulation of defense-related enzymes is examined, emphasizing the roles of pathogen recognition, phytohormone-mediated signaling pathways, transcriptional control, and signal cross-talk. Recent advances in omics technologies are highlighted as powerful tools for elucidating regulatory networks underlying enzyme-mediated defense responses. In addition to molecular mechanisms, this review explores the agricultural and biotechnological applications of defense-related enzymes, including their use as biochemical markers in breeding programs, targets for genetic improvement, and key components of induced resistance strategies. Current challenges, such as growth–defense trade-offs and environmental influences on enzyme activity, are also addressed, along with future perspectives for integrating molecular insights into sustainable disease management and crop improvement. Overall, this review underscores the importance of defense-related enzymes as major contributors to plant disease resistance and their potential contribution to resilient and sustainable agricultural systems.

Keywords: Plant immunity, Defense-related enzymes, Plant–pathogen interactions, Molecular regulation, Crop protection

*Corresponding author: Ondokuz Mayıs University, Faculty of Science, Department of Biology, 55139, Samsun, Türkiye

E mail: gonul.solmaz@omu.edu.tr (F. G. SEZGİN)

Fatma Gönül SEZGİN



<https://orcid.org/0000-0002-9400-5173>

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1. Introduction

Plant diseases caused by fungal, bacterial, and viral pathogens remain a major constraint on global agricultural productivity and food security. Yield losses associated with plant pathogens can reach substantial levels in many staple crops, posing serious economic and sustainability challenges (Savary et al., 2019; Shobade et al., 2025). Although chemical pesticides have been widely used for disease control, concerns regarding environmental pollution, human health risks, and the emergence of resistant pathogen strains have increased interest in plant-based, sustainable disease management strategies. Consequently, understanding and exploiting plant innate defense mechanisms has become a key focus in modern plant pathology and crop protection research. Plants have evolved a complex and multilayered immune system capable of recognizing and responding to pathogen invasion. This system involves both constitutive defenses and inducible responses that are activated upon pathogen perception. Early recognition of

pathogens through pattern recognition receptors (PRRs) triggers pattern-triggered immunity (PTI), while specific recognition of pathogen effectors leads to effector-triggered immunity (ETI), often resulting in stronger and more localized defense responses (Jones & Dangl, 2006). A central component of these immune responses is the activation of defense-related enzymes, which play pivotal roles in restricting pathogen growth and spread. Defense-related enzymes contribute to plant disease resistance through multiple biochemical and molecular mechanisms. These enzymes are involved in strengthening structural barriers, producing antimicrobial compounds, and generating reactive oxygen species (ROS) that function both as toxic agents against pathogens and as signaling molecules in defense pathways (van Loon et al., 2006). Among these, enzymes such as phenylalanine ammonia-lyase (PAL), peroxidases (POD), chitinases, β -1,3-glucanases, and polyphenol oxidase (PPO) have been extensively reported to be associated with enhanced resistance against a wide range of plant pathogens.



PAL is a key regulatory enzyme of the phenylpropanoid pathway, promoting the synthesis of lignin, flavonoids, and phytoalexins that reinforce cell walls and exhibit antimicrobial activity (Dixon et al., 2002). POD contribute to lignification, suberization, and oxidative burst responses, thereby restricting pathogen penetration and spread. In addition, chitinases and β -1,3-glucanases, classified as pathogenesis-related (PR) proteins, directly degrade fungal cell wall components and inhibit pathogen growth (van Loon et al., 2006). PPO further enhances defense by oxidizing phenolic compounds into toxic quinones, creating a hostile environment for invading pathogens.

At the molecular level, the activity of defense-related enzymes is tightly regulated by complex signaling networks involving phytohormones such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). These signaling pathways coordinate transcriptional and post-transcriptional regulation of defense enzyme genes, enabling plants to mount appropriate responses depending on the nature of the invading pathogen (Pieterse et al., 2012). Advances in molecular biology and omics technologies have further revealed that defense enzyme expression is influenced by epigenetic modifications, transcription factor networks, and cross-talk between signaling pathways, highlighting the dynamic nature of enzyme-mediated plant immunity.

Despite extensive research on individual defense enzymes, a comprehensive understanding of how these enzymes collectively contribute to disease resistance across different crop species and pathosystems remains fragmented. Moreover, translating enzymatic defense responses into practical applications for crop improvement and sustainable agriculture requires an integrated perspective that links molecular regulation with agronomic outcomes.

Therefore, this review aims to provide a comprehensive overview of defense-related enzymes involved in plant disease resistance, focusing on their biochemical functions, molecular regulation, and roles in plant-pathogen interactions. Additionally, the review discusses the potential applications of defense enzymes as biomarkers and targets in breeding programs and biotechnological strategies for developing disease-resistant crops, thereby contributing to sustainable agricultural practices.

Unlike previous reviews that primarily focus on individual defense enzymes or specific plant-pathogen systems, this review provides an integrated perspective on major defense-related enzymes by linking their biochemical functions with molecular regulation, signaling cross-talk, and practical agricultural applications. By incorporating recent advances from omics-based studies and crop improvement research, the review aims to define the current state of knowledge while highlighting emerging trends and future directions in enzyme-mediated plant disease resistance.

2. Overview of Defense-Related Enzymes in Plant Immunity

Defense-related enzymes are integral components of the plant immune system and are activated in response to pathogen perception. These enzymes function either directly by targeting pathogen structures or indirectly by strengthening host defenses. The coordinated action of multiple enzymes enables plants to mount robust and durable resistance against a wide range of pathogens.

3. Major Defense-Related Enzymes and Their Roles in Disease Resistance

3.1. Role of PAL in Plant Disease Resistance

PAL activity is rapidly induced in response to pathogen attack, mechanical injury, and elicitor treatment, highlighting its role as an early defense marker. Increased PAL activity has been consistently correlated with enhanced resistance to fungal, bacterial, and viral pathogens in various crop species. The phenylpropanoid-derived compounds synthesized downstream of PAL contribute to disease resistance through multiple mechanisms, such as reinforcing cell walls via lignification, producing antimicrobial phenolics, and modulating signaling pathways associated with plant immunity (Mandal et al., 2009).

Lignin deposition, a direct outcome of PAL-mediated phenylpropanoid metabolism, strengthens the plant cell wall and restricts pathogen penetration and spread. Similarly, phytoalexins synthesized through PAL-dependent pathways exhibit strong antimicrobial properties, inhibiting pathogen growth at infection sites. Studies in crops such as wheat, rice, tomato, and soybean have demonstrated that PAL induction is closely associated with reduced disease severity following pathogen challenge (Dixon et al., 2002; Mandal et al., 2009; Wu et al., 2025).

3.1.1. Molecular regulation of PAL

At the molecular level, PAL is encoded by multigene families in most plant species, allowing fine-tuned regulation in response to diverse biotic stresses. PAL gene expression is transcriptionally regulated by pathogen-induced signaling pathways involving salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). Among these, SA-dependent signaling has been particularly linked to PAL activation during defense against biotrophic and hemibiotrophic pathogens (Pieterse et al., 2012; Naz et al., 2024).

Transcription factors such as MYB, WRKY, and bHLH play crucial roles in regulating PAL gene expression by binding to cis-acting elements in PAL promoters. For instance, WRKY transcription factors activated during pathogen infection can directly enhance PAL transcription, thereby promoting phenylpropanoid biosynthesis and reinforcing defense responses (Vogt, 2010). In addition to transcriptional control, post-transcriptional and post-translational mechanisms, including mRNA stability and enzyme phosphorylation,

have been suggested to influence PAL activity under stress conditions.

3.1.2. PAL as a biomarker for disease resistance

Due to its rapid induction and strong association with resistance phenotypes, PAL has been widely proposed as a biochemical and molecular marker for disease resistance in plants. Elevated PAL activity has been used to evaluate the effectiveness of resistance inducers, biostimulants, and biological control agents. Moreover, PAL expression patterns are increasingly integrated into omics-based approaches, such as transcriptomics and metabolomics, to identify resistant genotypes in breeding programs (Mandal et al., 2009).

From an agricultural perspective, targeting PAL-mediated pathways offers promising opportunities for developing disease-resistant crops. Strategies such as marker-assisted selection, genetic engineering, and elicitor-based priming aim to enhance PAL activity and phenylpropanoid accumulation, thereby strengthening plant defense while reducing reliance on chemical pesticides.

3.2. Peroxidases (POD) in Plant Disease Resistance

Peroxidases (POD; EC 1.11.1.x) constitute a large and diverse group of oxidoreductase enzymes that play critical roles in plant defense against pathogen attack. These enzymes catalyze the oxidation of a wide range of substrates using hydrogen peroxide (H_2O_2) as an electron acceptor, thereby participating in reactive oxygen species (ROS) metabolism, cell wall reinforcement, and defense signaling (Hiraga et al., 2001). Due to their multifunctional nature, POD are considered important components of enzyme-mediated plant immunity.

3.2.1. Role of POD in defense responses

One of the earliest responses of plants to pathogen invasion is the rapid production of ROS, commonly referred to as the oxidative burst. POD contribute to both the generation and scavenging of ROS, allowing plants to balance antimicrobial activity with cellular protection. Elevated POD activity has been frequently associated with enhanced resistance to fungal and bacterial pathogens, as ROS can directly inhibit pathogen growth and act as secondary messengers in defense signaling cascades (Torres et al., 2006).

In addition to their role in ROS dynamics, POD are directly involved in cell wall fortification. POD-mediated lignification and cross-linking of cell wall polymers increase mechanical strength, creating a physical barrier that restricts pathogen penetration and spread. This structural reinforcement is particularly important in limiting the colonization of necrotrophic pathogens, which rely on tissue degradation for successful infection (Hiraga et al., 2001; Passardi et al., 2005).

3.2.2. Molecular regulation of peroxidase activity

Plant POD are encoded by multigene families, enabling diverse and stress-specific expression patterns. Pathogen-induced peroxidase expression is regulated at the transcriptional level through signaling pathways

involving salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). SA-dependent regulation is often linked to defense against biotrophic pathogens, whereas JA- and ET-mediated pathways are more commonly associated with resistance to necrotrophic pathogens (Pieterse et al., 2012).

Several transcription factors, including members of the WRKY and MYB families, have been shown to regulate peroxidase gene expression during pathogen challenge. Furthermore, post-translational modifications and changes in cellular localization influence POD activity, allowing rapid and localized defense responses at infection sites. The dynamic regulation of POD highlights their importance as flexible modulators of plant immune responses rather than static defense components.

3.2.3. POD as indicators of disease resistance

Due to their rapid induction and strong correlation with resistance phenotypes, peroxidase activity is widely used as a biochemical marker for plant defense activation. Increased POD activity has been reported in resistant cultivars of crops such as rice, wheat, maize, and tomato following pathogen infection. Consequently, POD assays are commonly employed to evaluate the effectiveness of resistance inducers, biological control agents, and biostimulants in experimental and applied agricultural studies (Passardi et al., 2005).

From an applied perspective, manipulating peroxidase-mediated pathways represents a promising strategy for crop protection. Enhancing POD activity through breeding, elicitor treatment, or genetic engineering can improve disease resistance while minimizing dependence on chemical pesticides. However, excessive ROS accumulation may lead to oxidative damage, emphasizing the need for balanced regulation of peroxidase activity in disease-resistant crop development.

3.3. Chitinases in Plant Disease Resistance

Chitinases (EC 3.2.1.14) are hydrolytic enzymes that catalyze the degradation of chitin, a major structural component of fungal cell walls and certain insect exoskeletons. In plants, chitinases are predominantly classified as pathogenesis-related proteins (PR proteins), specifically belonging to the PR-3, PR-4, PR-8, and PR-11 families. Due to their direct antifungal activity, chitinases represent a crucial enzymatic component of plant defense systems against fungal pathogens (van Loon et al., 2006; Grover, 2012).

3.3.1. Antifungal function of chitinases

The primary role of plant chitinases in disease resistance is the enzymatic hydrolysis of β -1,4-linkages in chitin polymers, leading to the weakening and lysis of fungal cell walls. This degradation not only inhibits fungal growth but also facilitates the release of chitin oligomers, which can act as elicitors to further activate plant immune responses (Grover, 2012). Consequently, chitinase activity contributes both directly and indirectly to enhanced disease resistance.

Numerous studies have demonstrated increased

chitinase activity in resistant plant varieties following fungal infection. Elevated levels of chitinase expression have been reported in crops such as rice, wheat, barley, tomato, and soybean in response to pathogens including *Fusarium*, *Rhizoctonia*, *Botrytis*, and *Alternaria* species (Kasprzewska, 2003; Grover, 2012). These findings underscore the importance of chitinases as key enzymatic barriers limiting fungal colonization and disease progression.

3.3.2. Molecular regulation of chitinase genes

Plant chitinases are encoded by multigene families, allowing differential regulation in response to developmental cues and environmental stresses. Pathogen-induced chitinase expression is regulated by complex signaling networks involving salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). Generally, SA-mediated signaling is associated with the induction of chitinases during defense against biotrophic pathogens, whereas JA and ET signaling pathways are more strongly linked to responses against necrotrophic fungi (Pieterse et al., 2012).

At the transcriptional level, chitinase gene promoters contain multiple cis-regulatory elements that interact with defense-related transcription factors such as WRKY and ERF proteins. Additionally, post-transcriptional regulation and differential subcellular localization contribute to the functional diversity of chitinases, enabling targeted defense responses at infection sites. Advances in molecular genetics have further revealed that chitinase expression can be enhanced through priming mechanisms, resulting in faster and stronger responses upon subsequent pathogen attacks.

3.3.3. Biotechnological and agricultural implications

Due to their potent antifungal activity, chitinases have been extensively explored as targets for biotechnological approaches aimed at improving disease resistance in crops. Transgenic plants overexpressing chitinase genes have shown enhanced resistance to fungal pathogens, confirming the functional importance of these enzymes in planta (Grover, 2012). Moreover, chitinase activity is frequently used as a biochemical marker to assess the effectiveness of resistance inducers, biological control agents, and elicitor-based treatments.

From an agricultural perspective, exploiting chitinase-mediated defense mechanisms offers a sustainable alternative to chemical fungicides. Integrating chitinase-related traits into breeding programs, either through conventional selection or molecular approaches, can contribute to the development of disease-resistant cultivars. However, the effectiveness of chitinases often depends on synergistic interactions with other defense enzymes, such as β -1,3-glucanases and POD, highlighting the importance of coordinated enzymatic defense networks.

3.4. β -1,3-Glucanases in Plant Disease Resistance

β -1,3-Glucanases (EC 3.2.1.39) are hydrolytic enzymes that catalyze the degradation of β -1,3-glucans, which are major structural polysaccharides of fungal cell walls. In

plants, β -1,3-glucanases are classified as pathogenesis-related proteins, primarily within the PR-2 family, and are widely recognized for their crucial role in defense against fungal pathogens (Balasubramanian et al., 2012; van Loon et al., 2006). Their activity represents a direct biochemical strategy to impair pathogen integrity and viability.

3.4.1. Antifungal activity and mode of action

The antifungal function of β -1,3-glucanases is mainly attributed to their ability to hydrolyze β -1,3-glucan polymers in fungal cell walls, leading to cell wall weakening, osmotic instability, and eventual lysis of fungal hyphae. This enzymatic degradation limits pathogen colonization and restricts disease development at early stages of infection. Additionally, the breakdown products of β -glucans can act as elicitors, further amplifying plant defense responses by activating downstream signaling pathways (Balasubramanian et al., 2012).

Elevated β -1,3-glucanase activity has been reported in resistant cultivars of several crops, including rice, wheat, barley, and tobacco, following infection by fungal pathogens such as *Fusarium*, *Rhizoctonia*, *Botrytis*, and *Magnaporthe* species. These observations highlight the importance of β -1,3-glucanases as core components of enzyme-mediated disease resistance.

3.4.2. Synergistic Action with Other Defense Enzymes

β -1,3-Glucanases often function synergistically with other defense-related enzymes, particularly chitinases, to enhance antifungal efficacy. While β -1,3-glucanases degrade glucan components of the fungal cell wall, chitinases target chitin polymers, resulting in a more comprehensive disruption of fungal structural integrity. This synergistic interaction has been shown to significantly improve resistance levels compared to the action of individual enzymes alone (van Loon et al., 2006).

Moreover, coordination between β -1,3-glucanases and POD contributes to both biochemical and physical defense barriers. The combined action of cell wall degradation and lignification effectively restricts pathogen spread and reinforces host resistance. Such enzyme networks emphasize that plant defense relies on integrated responses rather than isolated enzymatic activities.

3.4.3. Molecular regulation and agricultural relevance

The expression of β -1,3-glucanase genes is tightly regulated by pathogen-induced signaling pathways involving salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). Similar to other PR proteins, SA signaling is predominantly associated with β -1,3-glucanase induction during defense against biotrophic pathogens, whereas JA and ET pathways play more prominent roles in responses to necrotrophic fungi (Pieterse et al., 2012). Transcription factors such as WRKY and ERF proteins mediate the transcriptional activation of β -1,3-glucanase genes in response to pathogen challenge.

From an applied perspective, β -1,3-glucanase activity is frequently used as a biochemical indicator of plant defense activation. Transgenic approaches overexpressing β -1,3-glucanase genes have demonstrated enhanced resistance to fungal pathogens in several plant species, supporting their potential utility in crop improvement strategies. Furthermore, β -1,3-glucanases are increasingly considered promising targets in integrated disease management programs aimed at reducing chemical fungicide use and promoting sustainable agriculture.

3.5. Polyphenol Oxidase (PPO) in Plant Disease Resistance

Polyphenol oxidase (PPO; EC 1.14.18.1) is a copper-containing enzyme that catalyzes the oxidation of monophenols and o-diphenols to o-quinones. These highly reactive quinones subsequently polymerize to form brown pigments or interact with cellular macromolecules, leading to the formation of toxic environments that inhibit pathogen growth. PPO is widely distributed in higher plants and has been extensively implicated in defense responses against pathogens and herbivores (Mayer, 2006; Constabel and Barbehenn, 2008).

3.5.1. Role of PPO in defense against pathogens

The defensive role of PPO is primarily associated with the oxidation of phenolic compounds into quinones, which exhibit antimicrobial properties and can directly inhibit the growth of fungal and bacterial pathogens. Quinones can also cross-link with proteins and nucleic acids, reducing pathogen enzymatic activity and nutrient availability. Additionally, PPO-mediated reactions contribute to cell wall strengthening through the formation of phenolic polymers, thereby enhancing physical barriers against pathogen invasion (Mayer, 2006).

Numerous studies have reported increased PPO activity in resistant plant varieties following pathogen infection. Enhanced PPO activity has been observed in crops such as tomato, potato, wheat, and banana in response to pathogens including *Fusarium*, *Alternaria*, and *Xanthomonas* species. These findings suggest a strong correlation between PPO induction and reduced disease severity, supporting the role of PPO as an important component of enzyme-mediated plant defense.

3.5.2. Molecular regulation of PPO activity

PPO genes are typically encoded by small multigene families, and their expression is tightly regulated at both transcriptional and post-transcriptional levels. PPO activity is often compartmentalized within plastids, while phenolic substrates are located in the vacuole or cytosol. Upon pathogen attack or tissue damage, cellular compartmentalization is disrupted, allowing PPO to interact with phenolic substrates and initiate defense reactions (Constabel and Barbehenn, 2008).

The induction of PPO is regulated by defense-related phytohormones, particularly jasmonic acid (JA) and ethylene (ET), which are commonly associated with responses to necrotrophic pathogens and wounding. Salicylic acid (SA) signaling may also influence PPO expression depending on the host-pathogen system. Transcription factors involved in phenylpropanoid metabolism indirectly affect PPO-mediated defense by modulating phenolic substrate availability.

3.5.3. Agricultural significance and limitations

From an agricultural standpoint, PPO activity has been widely used as a biochemical marker for plant defense responses and resistance screening. PPO induction is often evaluated to assess the effectiveness of resistance inducers, biological control agents, and elicitor treatments.

Table 1. Examples of defense-related enzymes involved in plant disease resistance

Enzyme	Primary function	Crop examples	Target pathogens	Mode of action in defense	References
Phenylalanine ammonia-lyase (PAL)	Phenylpropanoid biosynthesis	Wheat, rice, tomato, soybean	<i>Fusarium spp.</i> , <i>Alternaria spp.</i> , <i>Pseudomonas spp.</i>	Lignin and phytoalexin synthesis; cell wall reinforcement	Dixon et al., 2002; Mandal et al., 2009
Peroxidases (POD)	ROS metabolism, lignification	Rice, wheat, maize, tomato	<i>Rhizoctonia solani</i> , <i>Xanthomonas spp.</i> , <i>Botrytis cinerea</i>	Oxidative burst; cross-linking of cell wall polymers	Hiraga et al., 2001; Passardi et al., 2005
Chitinases	Chitin hydrolysis	Rice, barley, tomato, soybean	<i>Fusarium spp.</i> , <i>Botrytis spp.</i> , <i>Alternaria spp.</i>	Degradation of fungal cell walls; elicitor release	Grover, 2012; van Loon et al., 2006
β -1,3-Glucanases	β -glucan degradation	Rice, wheat, tobacco	<i>Magnaporthe oryzae</i> , <i>Rhizoctonia solani</i>	Synergistic fungal cell wall degradation with chitinases	van Loon et al., 2006; Balasubramanian et al., 2012
Polyphenol oxidase (PPO)	Phenolic oxidation	Tomato, potato, banana, wheat	<i>Fusarium spp.</i> , <i>Xanthomonas spp.</i>	Quinone formation; toxic environment for pathogens	Mayer, 2006; Constabel and Barbehenn, 2008

Moreover, PPO-mediated defense mechanisms contribute to reduced pathogen colonization, thereby enhancing crop resilience under biotic stress conditions.

However, PPO activity also presents certain limitations in agricultural applications. Excessive PPO activity is associated with enzymatic browning, which can negatively affect the quality and marketability of fruits and vegetables. Therefore, while PPO contributes to disease resistance, its regulation must be carefully balanced to optimize both defense and crop quality. Understanding the molecular control of PPO expression and activity is essential for effectively exploiting its defensive potential in crop improvement programs.

A summary of major defense-related enzymes and their roles in plant disease resistance is presented in Table 1.

4. Molecular Regulation and Signaling Networks of Defense-Related Enzymes

The activation of defense-related enzymes in plants is controlled by complex and multilayered molecular signaling networks that link pathogen perception to transcriptional and biochemical defense responses. These regulatory networks ensure rapid induction of enzymatic defenses such as PAL, POD, chitinases, β -1,3-glucanases, and PPO in response to pathogen attack. Current understanding of these mechanisms is largely derived from molecular, genetic, and omics-based studies that elucidate how signaling pathways coordinate enzyme-mediated plant immunity (Jones and Dangl, 2006; Pieterse et al., 2012; Bigeard et al., 2015).

4.1. Pathogen Recognition and Early Signal Transduction

Plant immune responses are initiated through the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) localized at the plasma membrane, leading to the activation of pattern-triggered immunity (PTI). In cases where pathogens deliver effectors into host cells, intracellular resistance (R) proteins recognize these molecules and activate effector-triggered immunity (ETI). Both PTI and ETI trigger early signaling events, including calcium influx, activation of mitogen-activated protein kinase (MAPK) cascades, and rapid production of reactive oxygen species (ROS), which collectively function as upstream regulators of defense enzyme activation (Jones and Dangl, 2006; Bigeard et al., 2015).

These early signaling events promote transcriptional reprogramming and post-translational modification of defense-related enzymes, enabling plants to rapidly strengthen structural barriers and produce antimicrobial compounds.

4.2. Phytohormone-Mediated Control of Defense Enzymes

Phytohormones serve as central regulators of defense enzyme expression and activity, with salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) playing dominant roles in plant immune signaling. SA-mediated signaling is

primarily associated with resistance against biotrophic and hemibiotrophic pathogens and is closely linked to the induction of enzymes involved in phenylpropanoid metabolism, including PAL and specific peroxidase isoforms. SA signaling also promotes the accumulation of pathogenesis-related (PR) proteins such as β -1,3-glucanases, contributing to systemic acquired resistance (Vlot et al., 2009; Fu and Dong, 2013).

In contrast, JA and ET signaling pathways are predominantly activated in response to necrotrophic pathogens and mechanical damage. These pathways regulate the expression of enzymes such as chitinases, PPO, and certain POD that contribute to antifungal activity and oxidative defense. The coordinated action of JA and ET signaling enhances enzymatic degradation of pathogen cell walls and the production of toxic secondary metabolites (Pieterse et al., 2012).

4.3. Transcriptional Regulation of Defense Enzyme Genes

Transcription factors (TFs) act as key molecular nodes linking upstream signaling pathways to defense enzyme gene expression. Several TF families, including WRKY, MYB, ERF, and bHLH, have been extensively implicated in the regulation of defense-related enzymes. WRKY transcription factors bind to W-box elements in the promoters of numerous defense genes and play a central role in SA-mediated immune responses. Many genes encoding PAL, POD, and PR proteins are directly regulated by WRKY TFs during pathogen infection (Eulgem and Somssich, 2007).

MYB transcription factors are strongly associated with the regulation of phenylpropanoid metabolism and lignin biosynthesis, thereby influencing PAL activity and downstream defense pathways. ERF transcription factors integrate JA and ET signaling and regulate genes encoding chitinases and PPO, particularly during defense against necrotrophic pathogens (Liu et al., 2015).

4.4. Signal Cross-Talk and Fine-Tuning of Enzymatic Defense

The interaction between hormonal signaling pathways, commonly referred to as cross-talk, allows plants to fine-tune enzyme-mediated defense responses according to pathogen lifestyle and environmental conditions. Antagonistic interactions between SA and JA signaling pathways can suppress or enhance specific defense enzyme activities, ensuring that plants prioritize appropriate defense strategies. Conversely, synergistic interactions between JA and ET signaling can amplify the expression of enzymes involved in cell wall degradation and oxidative defense (Pieterse et al., 2012).

In addition to hormonal cross-talk, emerging evidence highlights the role of epigenetic regulation, including DNA methylation and histone modifications, in controlling defense enzyme gene expression. These mechanisms contribute to defense priming and stress memory, enabling plants to mount faster and stronger enzymatic responses upon recurrent pathogen exposure (Lämke and Bäurle, 2017).

4.5. Omics-Based Insights into Defense Enzyme Regulation

High-throughput omics approaches have substantially advanced the understanding of regulatory networks governing defense-related enzymes. Transcriptomic analyses reveal coordinated induction of enzyme-encoding genes following pathogen challenge, while proteomic studies provide insights into enzyme abundance, post-translational modifications, and subcellular localization. Metabolomic analyses further elucidate changes in phenolic compounds and other defense-related metabolites that serve as substrates for enzymatic reactions (Bigeard et al., 2015).

The integration of multi-omics datasets offers a systems-level perspective on enzyme-mediated plant defense and facilitates the identification of key regulatory components that can be exploited in crop improvement and disease resistance breeding programs.

5. Agricultural and Biotechnological Applications of Defense-Related Enzymes

Defense-related enzymes play a crucial role not only in natural plant immunity but also in modern agricultural and biotechnological strategies aimed at improving crop resistance to diseases. Understanding the molecular and biochemical functions of enzymes such as phenylalanine ammonia-lyase, POD, chitinases, β -1,3-glucanases, and PPO has enabled their practical exploitation in disease management, crop improvement, and sustainable agriculture.

5.1 Defense Enzymes as Biochemical Markers for Disease Resistance

The activity levels of defense-related enzymes have been widely used as biochemical markers for assessing plant resistance to pathogens. Enhanced activities of PAL, POD, chitinases, and β -1,3-glucanases are frequently correlated with reduced disease severity in resistant cultivars. As a result, enzyme activity profiling has become a valuable tool in plant breeding programs for early screening of disease-resistant genotypes (Hammerschmidt, 1999; Mandal et al., 2009).

Such biochemical markers offer advantages over traditional phenotypic screening, as they allow rapid and quantitative assessment of plant defense responses under controlled conditions. This approach is particularly useful for crops exposed to multiple pathogens and varying environmental stresses.

5.2. Genetic Engineering and Molecular Breeding Approaches

Advances in molecular biology have facilitated the manipulation of defense-related enzyme genes to enhance plant resistance. Transgenic plants overexpressing genes encoding chitinases, β -1,3-glucanases, or PAL have demonstrated increased resistance to fungal and bacterial pathogens in several crop species. These strategies directly strengthen enzymatic defense mechanisms or indirectly enhance

secondary metabolite production and cell wall fortification (Kasprzewska, 2003; Grover, 2012).

In addition to transgenic approaches, marker-assisted selection and genomic selection have enabled the incorporation of defense enzyme-related traits into elite cultivars. The identification of quantitative trait loci (QTLs) associated with enzyme activity further supports the integration of molecular breeding strategies for durable disease resistance.

5.3. Elicitors, Biostimulants, and Induced Resistance

Defense-related enzymes are central to induced resistance strategies that rely on the application of elicitors and biostimulants. Chemical elicitors such as salicylic acid analogs, jasmonates, and chitosan have been shown to stimulate the activity of key defense enzymes, leading to enhanced resistance against a broad spectrum of pathogens. Similarly, biological agents, including beneficial microorganisms and plant growth-promoting rhizobacteria (PGPR), activate enzyme-mediated defense pathways through induced systemic resistance (ISR) (van Loon et al., 2006; Walters et al., 2013).

The use of elicitors and biostimulants represents an environmentally friendly alternative to conventional pesticides and aligns with sustainable agriculture practices by reducing chemical inputs while maintaining crop productivity.

5.4. Balancing Defense and Crop Quality

Although the induction of defense-related enzymes is beneficial for disease resistance, excessive or uncontrolled enzyme activity may negatively impact crop quality. For example, high PPO activity is associated with enzymatic browning in fruits and vegetables, affecting post-harvest quality and marketability. Similarly, prolonged activation of oxidative enzymes may impose metabolic costs on plants, leading to growth penalties (Constabel and Barbehenn, 2008).

Therefore, biotechnological applications targeting defense enzymes must consider the balance between enhanced resistance and optimal plant performance. Fine-tuning enzyme expression through tissue-specific promoters, inducible systems, or genome editing technologies offers promising solutions to overcome these limitations.

6. Challenges and Future Perspectives

Despite substantial progress in understanding the role of defense-related enzymes in plant disease resistance, several challenges remain that limit their effective exploitation in agriculture. One major challenge is the complexity and redundancy of plant defense signaling networks. Defense enzymes are regulated by multiple pathways and are often influenced by environmental factors such as temperature, nutrient availability, and abiotic stresses, making it difficult to predict their behavior under field conditions (Pieterse et al., 2012).

Another limitation is the trade-off between growth and defense. Sustained activation of enzymatic defense responses may impose metabolic costs, leading to

reduced plant growth and yield. This trade-off is particularly relevant when defense-related enzymes are constitutively expressed or excessively induced, highlighting the need for precise regulation of enzyme activity in crop improvement strategies (Huot et al., 2014).

From a biotechnological perspective, the manipulation of single defense enzyme genes often results in partial or inconsistent resistance, as plant immunity relies on coordinated and multilayered defense mechanisms. Future research should therefore focus on network-based approaches that integrate multiple defense enzymes and signaling components. Advances in genome editing technologies, such as CRISPR/Cas systems, offer promising opportunities to fine-tune defense enzyme regulation without compromising plant fitness (Chen et al., 2019).

Additionally, emerging omics technologies and systems biology approaches are expected to play a pivotal role in unraveling the regulatory networks underlying enzyme-mediated defense. Integrating transcriptomic, proteomic, and metabolomic data will facilitate the identification of key regulatory nodes and enable the development of predictive models for disease resistance. Such insights will support the design of sustainable disease management strategies and the development of resilient crop varieties suited to changing environmental conditions.

Future research on defense-related enzymes should focus on integrative and systems-level approaches to better understand how multiple enzymatic pathways are coordinated during plant-pathogen interactions. Advances in genome editing technologies, multi-omics integration, and phenotyping under field conditions will enable more precise manipulation of enzyme-mediated defense responses. In addition, exploring the interaction between defense enzymes and environmental factors will be critical for translating molecular insights into durable disease resistance. Such efforts will facilitate the development of resilient crop varieties that balance enhanced defense capacity with optimal growth and productivity.

7. Conclusion

Defense-related enzymes constitute a fundamental component of plant disease resistance by mediating biochemical and structural defenses against a wide range of pathogens. Enzymes such as phenylalanine ammonia-lyase, POD, chitinases, β -1,3-glucanases, and PPO play complementary roles in reinforcing cell walls, generating antimicrobial compounds, and modulating oxidative stress during pathogen attack.

This review highlights the molecular regulation of these enzymes, emphasizing the importance of signaling networks, phytohormone cross-talk, and transcriptional control in shaping effective defense responses. Advances in molecular biology and omics technologies have significantly expanded understanding of enzyme-

mediated plant immunity and opened new avenues for their application in agriculture.

Future research aimed at integrating molecular insights with breeding and biotechnological approaches will be essential for harnessing the full potential of defense-related enzymes. A balanced and targeted manipulation of enzymatic defense pathways holds promise for enhancing crop resistance while maintaining productivity, thereby contributing to sustainable agricultural systems.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	F.G.S.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors declared that there is no conflict of interest.

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